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Heparin binding to lipoprotein lipase and low density lipoproteins

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Heparin was fractionated on an affinity column of bovine milk lipoprotein lipase (LpL) immobilized to Affi-Gel-15. The bound heparin, designated high-reactive heparin (HRH), enhanced LpL activity, presumably by stabilizing the enzyme against denaturation. The unbound heparin fraction had no observable effect on the initial rate of enzyme activity. However, at longer times of incubation there was inhibition of LpL activity. LpL-specific HRH also showed a high, Ca^{2+}-dependent precipitating activity towards human plasma low density lipoproteins (LDL). Since LpL and LDL both bind to heparin-like molecules at the surface of the arterial wall, we suggest that their similar heparin-binding specificity may have physiological consequences as it relates to the development of atherosclerosis.

Heparin binding *Lipoprotein lipase* *LDL* *Apolipoprotein* *Lipolysis*

1. INTRODUCTION

Lipoprotein lipase (LpL) is a rate-limiting enzyme in the removal of triglycerides from the circulation [1]. LpL is immobilized to the luminal surface of the capillary endothelia [2] by its interaction with specific cell membrane-associated heparin-like molecules [3–5]. LpL also binds to heparin-Sepharose and to heparin in solution [6–10]. In addition to LpL, triglyceride-rich lipoproteins and low density lipoproteins (LDL) also bind heparin [11–13]; the heparin-lipoprotein interaction is mediated by apolipoprotein B and apolipoprotein E [14]. Since both LpL and lipoproteins bind heparin-like molecules, the purpose of this study was to determine if a LpL-specific heparin also associates with lipoproteins. The results show that a LpL specific heparin also binds apolipoprotein B.

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2. MATERIALS AND METHODS

Sodium heparin (lot no. PM 10983, 154 IU/mg) from porcine intestinal mucosa was a generous gift from Hepar Industries, Franklin, OH. Bovine milk LpL was purified to homogeneity by chromatography on heparin-Sepharose by the method of Kinnunen [15] as modified by Socorro and Jackson [16]. LpL-Affi-Gel-15 was prepared as follows: 30 ml LpL (1.43 mg/ml) in 0.1 M Na phosphate, pH 7.5, 0.4 M NaCl, 0.5 M guanidine-HCl was added to 10 ml Affi-Gel-15 (Bio-Rad) equilibrated with 0.1 M Na phosphate, pH 7.5. After incubating overnight at 4°C with gentle mixing, the gel was incubated 4 h at room temperature with 2.5 ml of 1 M ethanolamine, pH 8.0, to block unreacted sites. The gel was then washed with 100 ml of 0.1 M $NaHCO_3$, pH 8.0, 2 M NaCl; 100 ml H_2O; and 100 ml of 10 mM Tris-HCl, pH 8.0, 0.15 M NaCl, respectively. The gel was stored at 4°C in 10 mM Tris-HCl, pH 8.0, 0.15 M NaCl, 0.01% NaN_3.

Heparin was quantitated by a modified car-

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bazole reaction procedure [17]. A turbidimetric assay was used to quantitate the heparin-mediated precipitation of LDL [4]; LDL were isolated from normal human plasma [18]. LpL activity towards *p*-nitrophenylbutyrate (PNPB) was determined by a modification of [19]. Time courses of the reaction were followed at 22°C by monitoring the production of *p*-nitrophenoxide (PNP) at 400 nm. The reaction mixtures contained 1 ml of 0.1 M Na phosphate, pH 7.25, 0.15 M NaCl, 0.5 mM PNPB, 8.45 μg LpL, and various amounts of heparin as indicated. Initial velocities were calculated from the slopes of the linear portion of each time course:

$$V_i\ (\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg LpL}^{-1}) = (10^6)\ \frac{(\Delta A_{400}/\text{min})(1\text{ ml}/1000\text{ ml})}{\epsilon\text{PNP (mg LPL)}}$$

where ΔA_{400}/min is the slope of the time course and ϵPNP is the absorptivity constant, 14775 $l\cdot M^{-1}\cdot cm^{-1}$. The increase in absorbance due to the spontaneous hydrolysis of PNPB was subtracted from the incubation which included enzyme.

3. RESULTS

Chromatography of crude heparin on a column of LpL immobilized to Affi-Gel-15 is shown in fig.1. After the sample entered the gel, the column was washed with 10 mM Tris-HCl, pH 8.0, 0.15 M NaCl, followed by 0.4 M NaCl; unbound heparin is designated unreactive heparin (URH). High-reactive heparin (HRH) was eluted with 1 M NaCl. Based on uronic acid content, <2% of the total heparin applied to the column was HRH. Heparin was not eluted with 1 M NaCl in control experiments performed with ethanolamine-substituted Affi-Gel-15. Rechromatography of HRH showed that >95% of the HRH uronic acid again bound to the affinity column and was eluted with 1 M NaCl (fig.1, inset).

To examine the specificity of URH and HRH for LpL, we next determined the effect of the isolated heparin fractions on enzyme activity with PNPB as substrate. As is shown in fig.2, addition of HRH to the incubation mixture enhanced LpL activity. The initial rate (fig.2, 100–180 s) of LpL catalysis in the presence of HRH was

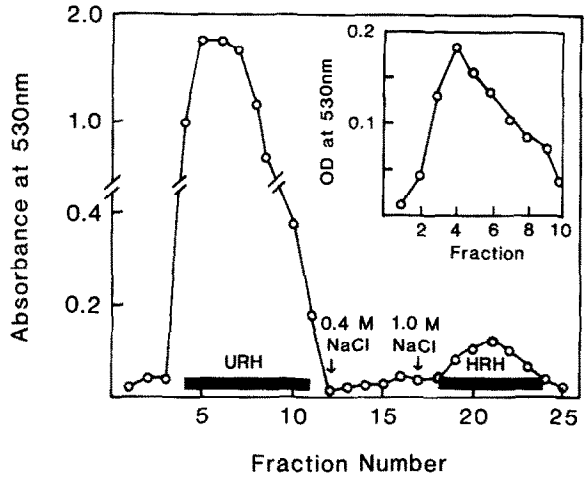

Fig.1. Chromatography of heparin on LpL immobilized to Affi-Gel-15. Heparin (150 mg) in 10 ml of 10 mM Tris-HCl, pH 8.0, 0.15 M NaCl was incubated with 5 ml LpL-Affi-Gel-15. The gel was poured into a column (2.5 × 9 cm) and eluted with the equilibration buffer. Heparin was eluted with 0.4 and 1.0 M NaCl, as indicated; 2-ml fractions were collected. Uronic acid (absorbance at 530 nm) was determined with 0.1 ml sample. Inset: HRH was rechromatographed over a second column of LpL-Affi-Gel-15 and eluted with 1 M NaCl as described above. The solid bars indicate the fractions that were pooled and used for subsequent experiments.

1.02 μmol·min^{-1}·mg LpL^{-1}. LpL catalysis in both the absence of heparin or presence of URH was 0.81 μmol·min^{-1}·mg LpL^{-1}. Moreover, the rates of catalysis decreased after 200 s, whereas in the presence of HRH the reaction was linear for up to 400 s. One possible explanation for the non-linearity of LpL catalysis in the absence of heparin is that LpL becomes denatured during the incubation. Fig.2 (inset) shows that in the absence of heparin LpL loses >50% of its activity after 20 min incubation. However, in the presence of HRH, LpL is stable for the full time scale of the enzyme assay.

It is known that heparin interacts with LDL to form insoluble complexes in the presence of Ca^{2+} [20]. Therefore, we next examined the binding of LpL-specific heparin and LDL with a turbidimetric assay. As shown in fig.3, HRH was 7-times more effective than URH in precipitating LDL; 2.2 μg HRH resulted in 50% precipitation of LDL whereas >15 μg were required for URH.

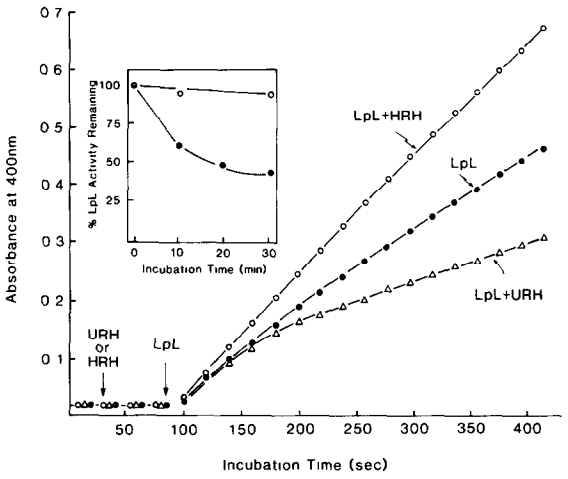

Fig.2. Effect of heparin on LpL catalysis. LpL activity was determined with PNPB as substrate as described in section 2. The incubation mixtures contained 0.5 mM PNPB, 4 μg URH or HRH, and 8.45 μg LpL in a final volume of 1.0 ml of 0.1 M Na phosphate, pH 7.25, 0.15 M NaCl. Inset: effect of preincubation of LpL on enzyme activity in the absence (●—●) and presence (○—○) of HRH. LpL was incubated at 37°C, and enzyme activity was determined at the times indicated.

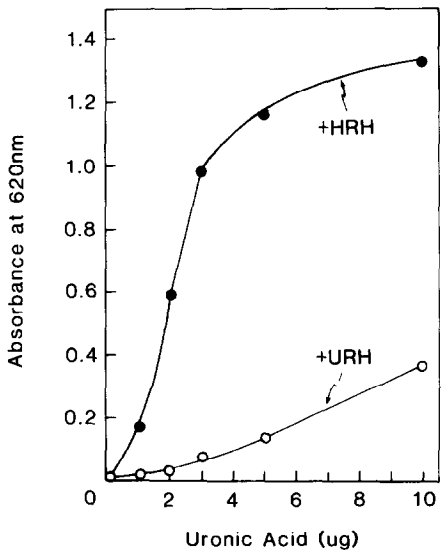

Fig.3. Precipitation of LDL by heparin. URH and HRH were incubated with LDL (150 μg protein) in 1.0 ml of 10 mM Tris-HCl, pH 8.0, containing 10 mM $CaCl_2$. After 5 min at 37°C, turbidity was determined by light scattering at 620 nm.

4. DISCUSSION

Matsuoka et al. [21] and Bengtsson et al. [22] previously described the isolation of LpL-specific heparin by affinity chromatography on immobilized LpL. However, in those reports the effect of the isolated fractions on LpL activity was not assessed. It is well known [23] that heparin stabilizes LpL activity. Our results show that the stabilization of LpL by heparin is specific for HRH. In contrast to HRH, URH inhibited LpL hydrolysis of PNPB. In this regard, Posner et al. [24] reported that high concentrations (1–2 mg/ml) of crude heparin competitively inhibit the LpL-catalyzed hydrolysis of trioleoylglycerol.

The binding of HRH to both LpL and LDL may be physiologically important as related to the development of atherosclerosis. It is well known that LDL binds to glycosaminoglycans within the arterial wall [25]. We speculate that LDL may compete with LpL for the same heparin-like receptors on endothelial cells, thereby reducing the amount of enzyme at the cell surface. A decrease in the amount of bound LpL could potentially lead to elevated levels of plasma remnant lipoprotein particles, which, in turn, may contribute to the process of atherogenesis [26].

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